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HEAT WORK AND PHOSPHORYLCREATINE BREAK-DOWN IN MUSCLE

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SUMMARY

1. A new instrument, the integrating thermopile, is described for measuring the total quantity of heat produced during muscular contraction.

2. This instrument has been used to investigate the relation between change of enthalpy ($-$(heat produced + work produced)) and break-down of phosphorylcreatine (ΔPC) in iodoacetate-poisoned frog sartorii at 0° C. In a variety of different types of contraction—series of isometric twitches, isometric tetani, contractions with positive and with negative work—the relation between enthalpy change and ΔPC was always the same, and corresponded to an *in vivo* molar enthalpy change (ΔH) of $-11{\cdot}0 \pm 0{\cdot}23$ (S.E.; $n = 52$) kcal/mole.

3. This value of ΔH is used to estimate the *in vivo* ΔH for ATP splitting and also the number of rephosphorylations to be expected per hexose unit oxidized by normal unpoisoned muscle.

INTRODUCTION

The possibility of linking biophysical and biochemical studies of muscular contraction is one that has attracted a revival of interest in recent years. The reasons for this, and the reasoning behind it, have been given before and need not be repeated here (see Carlson, Hardy & Wilkie, 1967). The present paper is an extension of the previous one; simultaneous measurements are made of physical energy output and of chemical break-down, and an attempt is made to overcome some of the difficulties that arose in the earlier work over the technique of heat measurement, and over statistical design. At the same time the scope of the earlier experiments has been extended to give more information about what happens when work is done by the muscle, or on it.

METHODS

All the experiments, unless otherwise stated, were made on frogs' sartorii (*Rana temporaria*) poisoned at 0° C with 0·5 mM iodoacetate and nitrogen. The methods of handling the muscles, of analysing them for phosphorylcreatine (PC) by the Ennor & Rosenberg method and of calculating the results, were exactly as described before (Carlson, Hardy & Wilkie, 1963; Dydyńska & Wilkie, 1966; Carlson *et al.* 1967). However, a new technique has been developed for measuring heat production. An outline description has already been given (Wilkie, 1963) and this technique was used in the thermochemical studies by Dydyńska & Wilkie (1966), but this is the first description in detail.

Measurement of heat production

It has become necessary to devise new instruments for measuring the heat production of muscles for several reasons. The most pressing is that Mr A. C. Downing has retired and the thermopiles that he constructed are progressively becoming unusable because of failure of their mica insulation. These thermopiles had been constructed to be as thin as possible and as sensitive as possible so as to suit them for the moment-to-moment analysis of heat production which is Professor A. V. Hill's main field of interest. However, quite apart from the difficulties of construction, such thermopiles are not ideally suited for thermochemical experiments in which it is vital to know, in absolute terms, the total amount of heat liberated in the whole of the muscle—for the whole of the muscle is analysed chemically—but fast time-resolution is, at present, of secondary importance.

It seemed that three aspects of the established design could be improved to make it more suitable for the present purpose:

1. *Integration.* As A. V. Hill has often emphasized, we cannot safely assume that the rise of temperature is the same at all points in a muscle. Slight inequalities of strength can lead to marked non-uniformities in temperature, especially when the muscle is being stretched either while it is active or while it is relaxing. Hill–Downing type thermopiles (see Hill, 1965), especially if they are equipped with a protecting region, can only sample the temperature in a limited length of the muscle. What is really needed is a modification that integrates the heat production over the whole volume of the muscle.

2. *Absolute calibration.* In order to convert units of temperature rise into those of heat, it is necessary to multiply by the heat capacity of the thermopile plus muscle and adherent Ringer solution. This quantity cannot be determined with high accuracy; and the uncertainty is particularly great in experiments like the present ones in which a single muscle, sometimes a small one, is applied to the pile. Some alternative means of absolute calibration is therefore desirable.

3. *Reduced correction for heat loss.* In series of twitches or long tetani, such as are employed in the present experiments, it is vital to correct for the heat that has been conducted away from the muscle in order to arrive at an accurate total. Since on a classical thermopile (e.g. D4) the temperature of a single 70 mg muscle typically falls with a time constant of 10 sec, this correction for heat loss soon becomes an embarrassingly large fraction of the total. Reduction in the thermal conductivity away from the central region would improve this situation.

The integrating thermopile (Wilkie, 1963) attends to these three features, though at the expense of decreased speed of response. It resembles a calorimeter opened out flat so that the muscle can lie on its surface. As shown in Fig. 1*A*, the muscle lies on a plastic-covered strip of silver—the central shaded area—which is roughly the same size and shape as the muscle, and is 250 μ thick. The high thermal diffusivity of silver ensures that longitudinal temperature gradients are rapidly equalized, while conduction from the muscle into the silver is rapid because of the short distance involved. Since, in addition, thermal conduction

away from the central strip is very slow, the whole central area rapidly reaches a uniform temperature to which all parts of the muscle have contributed. The rise of temperature is measured by two chromel–constantan junctions in the position shown; they give an ample signal-to-noise ratio for the tasks so far attempted, e.g. 50:1 in a single isometric twitch.

The requirement for absolute calibration independent of muscle weight is met by equipping the silver strip with a fine insulated constantan wire which runs in a groove in the silver. At each end the constantan is joined to two fine copper leads. By passing known currents down two of the leads and measuring the potential difference across the other two, it is easy to determine accurately the resistance (about 5 Ω) of the constantan wire. It is also possible to confirm experimentally that currents passing in the copper leads do not

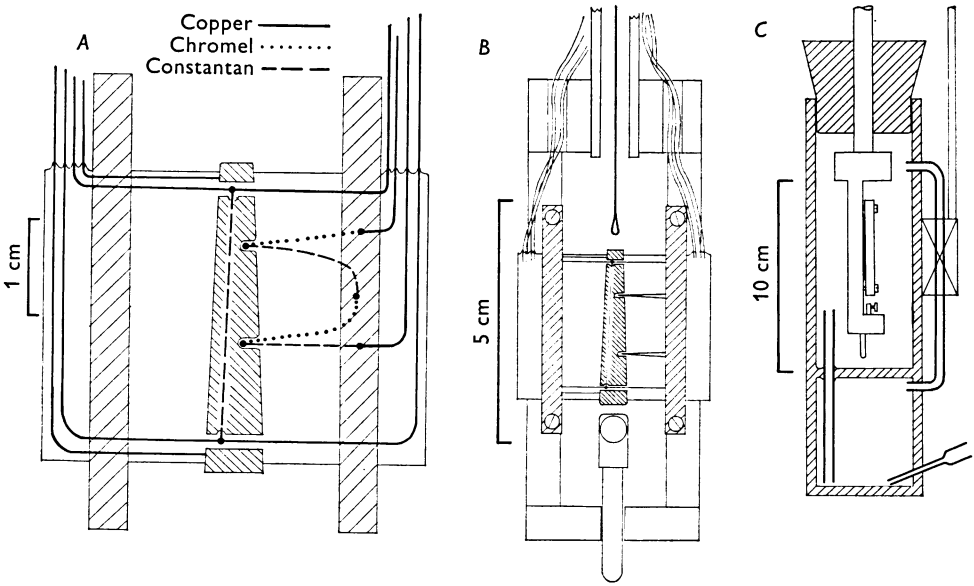

Fig. 1. The integrating thermopile. The scale of the diagram is shown on the left in each case.

(*A*) Face view showing arrangement of wires. Continuous lines, copper; dotted lines, chromel; dashes, constantan.

(*B*) Face view showing thermopile element clamped in frame.

(*C*) Side view showing arrangement of stainless-steel outer chamber. The regulating valve is shown diagrammatically on the right-hand side; it is operated by a rod which projects upwards out of the thermostat. Ringer fluid is not shown: a stream of O_2 or N_2 enters by the jet at the lower right-hand corner.

appreciably heat the central strip, which would lead to errors in calibration. Moreover, by connecting the heating assembly to a galvanometer, and thus employing the copper–constantan joints as thermojunctions, it is possible to measure directly the temperature difference between tibial and pelvic ends of the muscle.

The rate of heat loss from the central region is kept to a minimum by mounting the silver strip on a thin support of mica and plastic and keeping the metallic connexions to the periphery as thin and as few as possible. A low rate of heat loss compared with the rate of internal temperature equalization is essential if the temperature within the central region is to remain fairly uniform.

Practical details

Referring to Fig. 1, the thermojunctions and calibrating wire are made from 40 or 42 s.w.g. (122 or 102 μ) wire, with joints brazed in the small gas flame from a fine hypodermic needle. Electrical insulation of the whole assembly, which must withstand prolonged immersion in Ringer fluid, proved to be something of a problem. At first the silver and wires were sandwiched between two sheets of mica, the spaces being filled with Araldite (epoxy, Ciba) resin. This provided many disappointments because the mica fails to adhere or perhaps splits from the exposed edge. Once water has penetrated between two layers of mica they separate almost as easily as do the pages of a book. More recently a single sheet of mica has been used as a mechanical foundation for the assembly and the insulation has been supplied by a plastic wrapping of Saran (PVDC copolymer, Dow Chemical Co., 16 μ thick) or, better still, Melinex (polyester, I.C.I., 10 μ thick) fixed with Araldite. This seems to work satisfactorily without adding much to the total thickness. The plastic film also covers the region at both sides where soldered joints are made to the fine polyvinylchloride-covered flexible leads. It is essential that all these leads should be made from the same length of wire because the leads are subjected to a large temperature gradient and remarkably large thermo-electric effects appear between apparently similar batches of copper wire.

The thermopile element forms an independent unit which is then, as shown in Fig. 1*B*, clamped in a metal frame in such a way that the reference junctions are in close contact with a large heat sink. After some experience with gold-plated brass and gold-plated aluminium frames, the best material was found to be anodized aluminium, which seems to be resistant to salt water. The tubular stem leading up to the mechanical recording apparatus is made of fibreglass (Bantex Ltd.) to minimize heat conduction.

The thermopile and frame are enclosed within a stainless-steel outer cover arranged as shown in Fig. 1*C*. When the side tube is clamped, the Ringer is driven up by the entering gas and bathes the muscle; when the side tube is opened the Ringer sinks down clear of the thermopile and the upper chamber fills from the lower chamber with gas whose temperature and water vapour content should have had ample time to come to equilibrium.

Thermopile and cover are in turn submerged in a large Dewar vessel containing ice and water and stirred by blowing in pre-cooled air. These arrangements for thermostatting are at present one of the least satisfactory features of the technique. Only after very prolonged immersion, preferably without the outer jacket, does the output of the thermopile fall to zero. In general use, and especially in these chemical experiments where repetition is a statistical necessity, prolonged equilibration is impracticable and the thermopile produces a residual voltage because of the residual temperature gradients within it. Various modifications such as the use of crushed ice with only a little water, or the replacement of the bubbles by a mechanical stirrer, have not produced a conclusive improvement; the residual voltages have an elusive quality which makes them very difficult to investigate.

The output from the thermopile goes directly to an all-copper reversing switch (Tinsley and Co.). This is essential if an absolute base line is to be maintained; for only by reversing the circuit at this point can the potential produced by the thermopile be distinguished from those potentials that arise elsewhere in the non-thermostatted parts of the input circuit. From the reversing switch the circuit passes via a shift and calibration circuit to a Downing galvanometer (period 38 msec, resistance 18 Ω, resistance for critical damping about 250 Ω) with photo-electric amplification slightly modified from that devised by A. V. Hill (see Hill, 1965). The low-resistance input circuit is very sensitive to magnetic interference whose effects are minimized by avoiding loops (e.g. by running one conductor inside the other). Where loops are inevitable as in the calibrating circuit and the reversing switch, interference is diminished by making the circuits physically small and by enclosing them in mumetal boxes. The end result of these precautions is that the total noise level is about 0·006 μV peak-to-peak under ordinary working conditions. This is roughly double the Brownian noise to be expected in

the galvanometer (see Hill, 1965, p. 276). When used at maximum sensitivity the galvanometer is heavily over-damped by the low external circuit resistance of approximately 5 Ω: its response is then exponential with a time constant of 0·17 sec. When large enough signals are available the response of the galvanometer can be speeded up and the linearity improved by applying negative feed-back.

Method of use

The amplified output from the two chromel–constantan junctions is finally displayed on a Sanborn Model 322 recorder. With a muscle on the thermopile the absolute calibration is obtained by passing a current through the calibrating wire and adjusting it until a steady deflexion is obtained, as shown in Fig. 2*A* (1) to (2). Measuring the current I A, the rate of heat production in the calibrating wire resistance, R Ω, is $I^2R \times 0{\cdot}239$ cal/sec. If the steady deflexion were h_0 scale divisions, the calibration number *for steady deflexions only* would be simply $0{\cdot}239 \times I^2R/h_0$ cal/sec for each scale division. However, for most purposes we are interested not in the *rate* of heat production, but in the *total amount* of heat contained in the central area over and above what it contained when it was all at ambient temperature. This second calibration number is obtained by switching off the calibrating current, whereupon the temperature of the central area falls exponentially ((2) to (3)) with a time constant, which can be determined from the record, of τ sec. It then follows that each scale division represents $\tau \times 0{\cdot}239 \times I^2R/h_0$ cal of heat still remaining in the central area. Note that the calibration procedure does not demand knowledge of the thermal capacity of muscle and thermopile, or of the sensitivity in μV/deg. of the thermojunctions.

Theory

It is assumed that the muscle and the central area of the thermopile, of thermal capacities M and P cal/deg. respectively, are at uniform temperature θ degrees above ambient. The (excess) heat content, Q cal, which we wish to determine is

$$Q = \theta(M+P). \tag{1}$$

Other symbols that will be used are:

C the coefficient of heat loss from the central region, cal/deg. × sec;

V the overall sensitivity of the recording apparatus, scale divisions/μV;

Z the thermo-electric coefficient of the thermojunctions μV/deg.;

t the time in sec.

During the calibration period the central area is heated to a steady temperature θ_0 with corresponding deflexion h_0 scale divisions. Since the rate of heat production must then equal that of heat loss

$$0{\cdot}239 \times I^2R = C \times \theta_0. \tag{2}$$

When the heating current is switched off, the temperature falls and

$$\mathrm{d}\theta/\mathrm{d}t = -C \times \theta/(M+P)$$

so that

$$\theta/\theta_0 = h/h_0 = \exp(-tC/(M+P)).$$

The temperature falls exponentially with time constant τ

$$\tau = (M+P)/C, \tag{3}$$

which can be determined from the experimental record.

For a given deflexion h

$$Q = \theta_0 (M+P) \times h/h_0 \quad \text{from (1);}$$

substituting from (2) and (3) so as to eliminate C and θ_0

$$Q = \tau \times 0{\cdot}239\ I^2R \times h/h_0$$

which is the expression that we require.

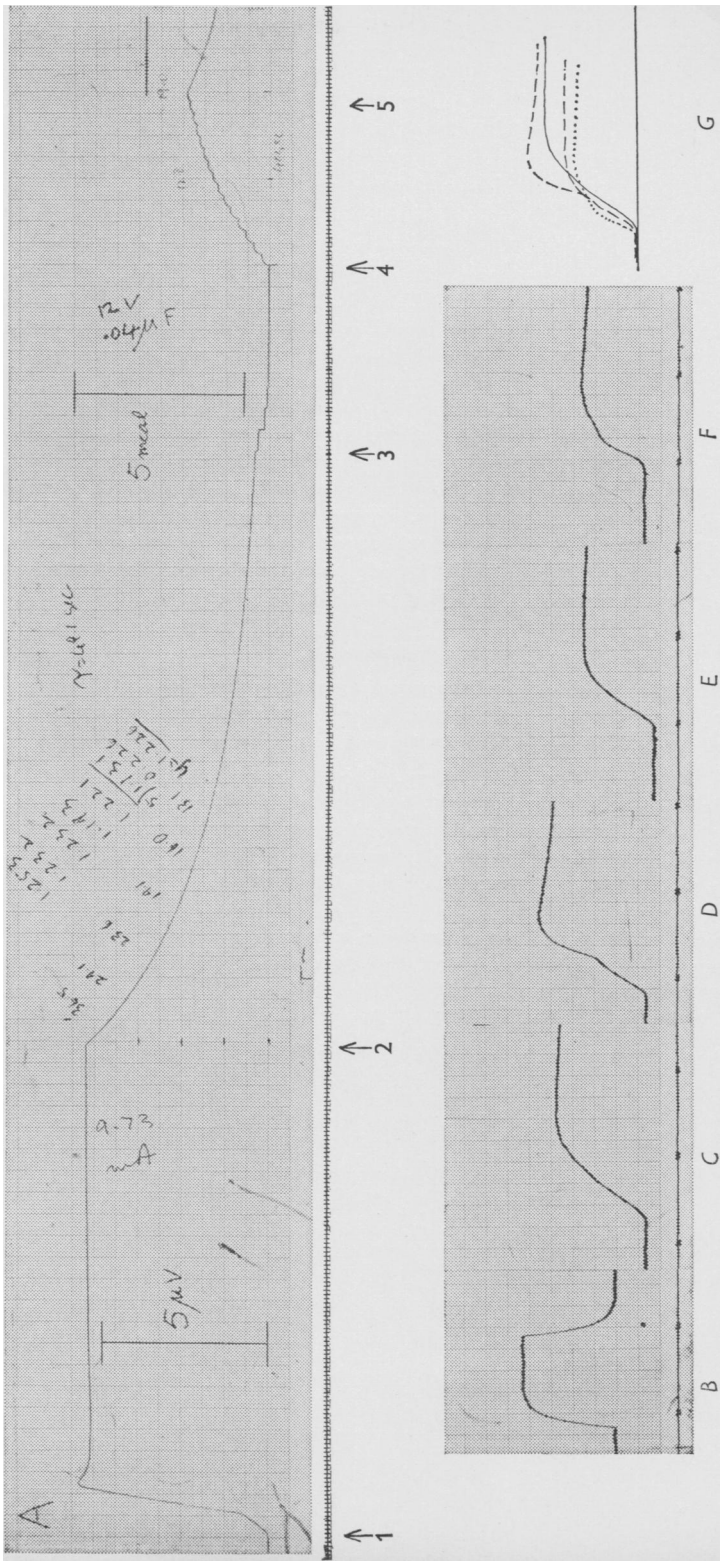

Fig. 2. For legend see opposite page.

It is of some value to calculate in addition the value of C, the coefficient of heat loss. Substituting h_0/VZ for θ_0 in equation (2) and rearranging

$$C = V \times Z \times 0{\cdot}239\, I^2R/h_0$$

Z can be measured experimentally on junctions similar to those employed in the thermopile. The result, 116 μV/deg., is exactly equal to what would be expected for two chromal constantan junctions; so it can be used for experimental determination of C. This is useful for three purposes:

(1) *Checking measurements and calculations.* For a given thermopile, C is found to be reasonably constant. For example, with fifty-two different muscles of widely varying weight on thermopile W6 (the one used in this investigation), C was $2{\cdot}195 \pm 0{\cdot}114$ mcal/deg. $\times$ sec (mean $\pm$ S.D.). A large departure from this value indicates that a mistake has been made somewhere.

(2) *Calibration without applying a measured current.* By combining the previous two equations, eliminating I^2R and rearranging:

$$Q = h \times C\tau/VZ.$$

Thus Q can be calculated from h with reasonable accuracy if τ is known.

(3) *Calculation of thermal capacity of thermopile, P.* Rearranging equation (3):

$$P = \tau \times C - M.$$

The values of M can be estimated from the frozen weight of the muscles, P then comes out at the equivalent of $40{\cdot}5 \pm 10{\cdot}8$ mg of muscle (mean $\pm$ S.D., $n = 52$), about $1\frac{1}{2}$ times as much

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Legend to Fig. 2.

Fig. 2. Typical records to illustrate the performance of integrating thermopile W.6. Time marks every sec.

(*A*) Complete record of an experiment on thirty isometric twitches of an IAA poisoned muscle, to illustrate calibrating procedure. At (1) the heating current was switched on and adjusted by hand until the deflexion was steady: the current was then 9·73 mA. After switching off the current (2) the curve fell exponentially as shown by the almost constant ratio y between successive ordinates at 10 sec intervals (numbers obliquely above). The time constant τ is calculated from $\tau = 10/\log_e \bar{y}$ sec, where $\bar{y}$ is the mean value of y. In this case $\bar{y} = 1{\cdot}226$ and $\tau = 49{\cdot}1$ sec. The heat calibration is then calculated as explained in the text.

From (3) the recorder was run for a few sec at 1 min intervals until the record had returned to its base line. Between (4) and (5) the muscle was stimulated by 15 condenser discharges (12 V, 0·04 μF) at roughly 3 sec intervals. The rising curve shows the output of the thermopile and the horizontal bar the final deflexion after correction for heat loss.

(*B*)–(*G*) Records of single twitches from a pair of normal muscles at 0° C to show performance at higher speed and sensitivity. Noise level 0·007 μV p-p.

(*B*) Voltage calibration 0·324 μV. There was sufficient feed-back to reduce sensitivity to 0·54 of the open-loop value. This reduced the time constant of response of the galvanometer from 0·17 to 0·09 sec so that the main limitation was heat conduction and equalization.

(*C*) Isometric twitch.

(*D*) Isotonic twitch, load 0·3 P_{max}. The hump is due to energy dissipation when the load falls.

(*E*) shows that there is no hump in a similar contraction where the load is held up at the peak of shortening.

(*F*) Isotonic twitch, load 0·02 P_{max}.

(*G*) Superimposed tracings of *C* (continuous), *D* and *E* (dashes) and *F* (dots).

as the value calculated for the silver, mica, etc., immediately underlying the muscle. This may be because the structures surrounding the central area must warm up when the central area does so and thus they contribute something to the total thermal capacity.

The observed value of C, the coefficient of heat loss, is roughly 10 times larger than it would be if the heat was conducted away only by passing sideways in the solid material of the support and wires. Presumably the remainder is lost by conduction to the air, evaporation and perhaps radiation. An attempt to disentangle these factors by measuring C *in vacuo* proved inconclusive (C fell to about half its previous value) because the plastic materials of the thermopile produced so much outgassing that the pressure could not be reduced convincingly below 10^{-3} mm Hg; thus the thermal conductivity of the remaining air was probably still appreciable.

Correction for heat loss

The quantity Q cal that has been calculated above is the amount of heat remaining in the thermopile and muscle at the moment in question (time = t) when the deflexion is h scale divisions. Normally we wish to know the quantity of heat that has been produced up to this moment, so we must add on to h a correction for the heat lost, which amounts to

$$\frac{1}{\tau}\int_0^t h.\mathrm{d}t \text{ scale divisions.}$$

The integral can be obtained numerically, using a trapezoidal approximation (see Hill, 1965, p. 314) or Simpson's rule, which gives remarkably accurate results with very few values of the ordinate. Recently the correction has been made electronically.

Tests of performance

The general character of the records produced is shown in Fig. 2. No detailed tests were made on the speed of heat conduction from muscle to thermopile, since this instrument is not designed for the analysis of rapid events. A rough indication of speed is given by the fact that impulsive heating of an agar 'muscle' by a condenser discharge leads to a roughly exponential rise in temperature with a time constant of about 0·7 sec. Nevertheless, as Fig. 2, *B–G* shows, it is possible to make out some of the familiar features of the time course of heat production in a single twitch. For example, the smaller the load and the faster the shortening, the greater is the rate of heat production (shortening heat). When a load is allowed to fall during relaxation, work is dissipated into heat within the muscle. When work is done, extra energy is mobilized by the muscle (Fenn effect): the difference in final level of the three lower curves in *G* is largely accounted for by the varying amounts of internal work done: if the work is subtracted in each case, the residue is roughly constant (see Carlson *et al.* 1963).

However, it was really important that the new method of absolute calibration should be checked independently, since this problem has proved troublesome in the past (see Hill & Woledge, 1962). The theory seemed satisfactory but there was no way of knowing to what degree the actual thermopile approximated to the physical system envisaged.

Numerous attempts were made to liberate accurately known quantities of heat in 'muscles' of agar or chamois leather by discharging condensers into them but the results were always erratic, presumably because of unpredictable effects at the electrodes and in the electrolyte. The final solution was to use the central part of a resistance strain gauge (Tinsley type 6B, 604 Ω) whose wire grid was of similar size (6 × 31 mm) and shape to a muscle. This was stuck on the face of the thermopile with petroleum jelly. Known amounts of energy were dissipated in it either impulsively or continuously and, as Fig. 3 shows, the deflexion produced was in all cases close to what would be expected from the usual, and completely independent, calibrating procedure.

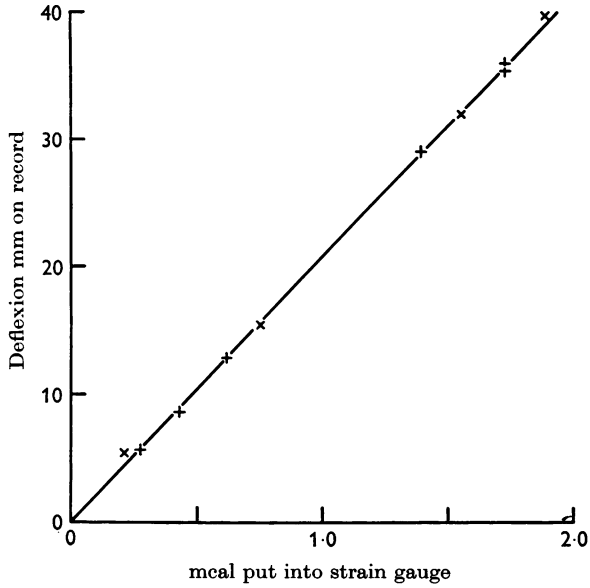

Fig. 3. Test of calibrating procedure by dissipating known amounts of energy into a 604 Ω resistance strain gauge stuck on the surface of the thermopile. Ordinates: deflexion, mm on record. Abscissae: energy dissipated in strain gauge, mcal. Vertical crosses: impulsive heating, 1 μF, various voltages. Diagonal crosses: continuous heating, various currents for 1–2 sec. Line: calibration derived independently from the normal procedure using internal heating wire.

RESULTS

As explained in the Introduction, the aim of these experiments was to explore the relation between energy production in the form of heat and work, and break-down of phosphorylcreatine (ΔPC) in types of contraction that differed from each other as much as possible. Four types of contraction were investigated: (1) Series of isometric twitches. (2) A single isometric tetanus. (3) A series of after-loaded isotonic twitches: work performed *by* the muscle. (4) Slow isotonic stretch during tetanus: work performed *on* the muscle.

1. Series of isometric twitches

The muscle was stimulated every 3 sec so that there was ample time for complete relaxation between responses. In fifteen experiments the number of twitches was kept constant at thirty, so as to provide a direct basis for comparison with the earlier work of Carlson *et al.* (1967), also on series of thirty twitches.

Experiments with thirty twitches. When the amount of activity is kept

constant, variation in energy production and ΔPC arises solely from variation in size between one muscle and another. In order to examine the relationship over as wide a range as possible, muscles were deliberately chosen with a wide range of weights, from 55·6 to 124·0 mg (frozen weight). The results are shown in Fig. 4 from which it is clear that heat (h) and ΔPC are strongly correlated (r = 0·89). Both regression lines are shown,

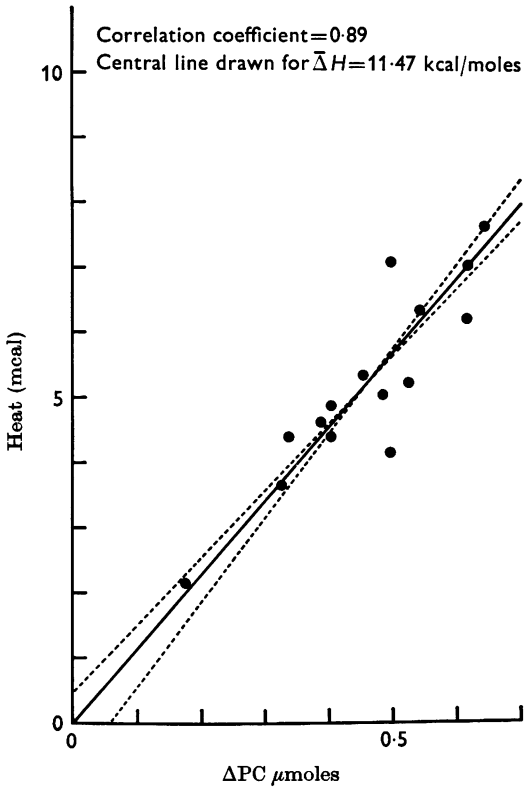

Fig. 4. The relation between heat produced (mcal, ordinates) and phosphoryl-creatine break-down (μmole, abscissae) in sets of thirty isometric twitches. The dotted lines indicate the two regression lines: the continuous line has been drawn with a slope of 11·47 kcal/mole as described in the text.

whose slopes are 10·3 and 13·1 kcal/mole and whose intercepts fall more or less symmetrically to either side of zero. Since both variables undoubtedly contain errors, the true functional relation passes closer to the origin than either regression line does; indeed there is no reason to doubt that it actually passes through the origin. Assuming that this is the case, it is legitimate to calculate the individual ratios (heat/ΔPC) and to derive their mean and its standard error, 11·47 ± 0·36 kcal/mole (n = 15).

Experiments with other than thirty isometric twitches. In order to explore

the relation between heat and ΔPC over a wider range than could be achieved by choosing muscles of varying size, twelve other experiments were performed in which the number of twitches was varied from 4 up to 107. The results are shown in Fig. 5 by open circles; the filled circles are those previously plotted in Fig. 4.

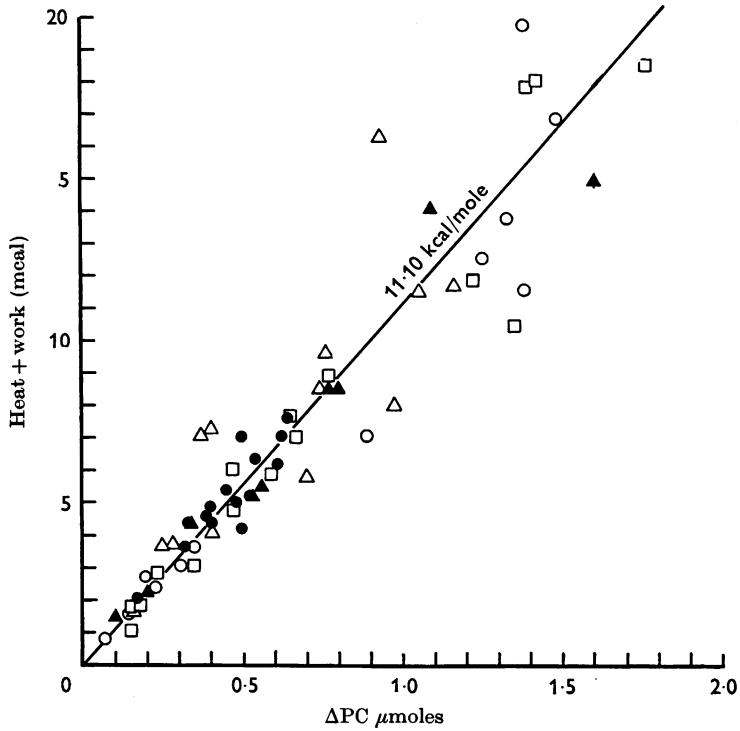

Fig. 5. The relation between heat, work and PC splitting in various types of contraction. Ordinates: (heat produced + work produced) by muscle, equal to minus (change of enthalpy of muscle), mcal. Abscissae: phosphorylcreatine split, ΔPC, μmoles. ○ From 4 to 107 isometric twitches; 12 experiments. ● 30 isometric twitches; 15 experiments. □ Isometric tetani lasting from 7 to 111 sec; 16 experiments. ▲ From 8 to 78 isotonic twitches with performance of positive mechanical work; 9 experiments. △ From 2 to 8 tetani, duration seven sec, with slow isotonic stretch and negative work; 13 experiments. The line has been drawn with slope of 11·1 kcal/mole for reasons explained in the text.

2. Single isometric tetani

Sixteen similar experiments were performed on tetani whose duration varied from 7 to 111 sec. The frequency of stimulation was kept low (5/sec) so as to avoid confusion from dissipation of the energy in the stimulus itself. Even in the case of the longest tetanus, the correction for energy in the stimulus amounted to only 2 % of the total heat liberated. No doubt

the tetani were not quite completely fused, but the variation in tension was not great enough (< 1 %) to show up with the recording sensitivity that was used. The results are shown in Fig. 5 by squares.

3. Series of isotonic twitches with performance of positive work

Results shown in Fig. 5 by black triangles. The load was lifted then allowed to fall again. The kinetic energy in the lever and load is small so that the total energy production was measured in the form of heat in the muscle. The aim in these nine experiments was that as large as possible a fraction of the total energy should once have existed as mechanical work. This necessitated that the experiments should be performed with series of twitches or short tetani, and that the load should be optimal for the size of muscle. In order to adjust the load, each muscle's isometric twitch tension was measured before poisoning with iodoacetic acid (IAA). The muscle was then allowed to rest for 40 min in oxygenated Ringer solution so as to rebuild the PC that had been broken down (see Dydyńska & Wilkie, 1966, Fig. 1). The isotonic load was set at 50 % of the isometric tension, since the muscle then gives roughly the maximal amount of work. In the long series of twitches it might actually have been better to use a somewhat smaller load, to compensate for the fall in tension development towards the end of the series of twitches. Even so, the external work made up an appreciable fraction of the total energy, 30 % on the average. An allowance for the work done internally in stretching the series elastic element would bring this figure up to about 35 %. Since the contractions in each set were all against the same load, and all of them involved the performance of work, it is not possible to say accurately that this work appears as an extra term in the energy production, in the way described by Fenn (1923, see also Carlson *et al.* 1963). However, the total energy production per twitch certainly was about 25 % greater in these isotonic contractions than in isometric ones. This is roughly equal to the difference in (external + internal) work production in the two cases. Moreover, other experiments have shown that muscles poisoned with IAA, like those poisoned with fluorodinitrobenzene (FDNB) (Dydyńska & Wilkie, 1966) do show a normal Fenn effect.

4. Stretches applied to the active muscle: negative work

In past work on the heat production of muscles subjected to stretches, two rather different techniques have been employed. Abbott, Aubert & Hill (1951) and later Hill & Howarth (1957) employed quick stretches applied with a Levin-Wyman lever; while Abbott & Aubert (1951) stretched the muscle slowly using a constant load greater than the isometric tension. The greatest effect of stretch on energy output was that reported

by Hill & Howarth, who applied fairly rapid stretches during a muscle twitch. However, their technique was not followed in the present experiments, for two reasons.

First, the amount of work done on the muscle is large, hence the quantity of interest (heat output − work done on the muscle) is a small difference between two large quantities one of which, the heat, is rather uncertain because of the likelihood that the heat production is non-uniform during such stretches. Admittedly, this problem should be less severe using the integrating thermopile than a traditional one, but it seemed wiser to avoid it if possible.

Secondly, in order to accumulate a reasonably large total quantity of chemical break-down, and also to obtain a fair spread of points, a number of twitches would be necessary. Before each twitch with a stretch it would be necessary to include one without stretch, so as to make certain that the muscle had returned to its original length. This would considerably dilute the effect being examined.

Instead, a technique similar to that of Abbott & Aubert (1951) was adopted: the muscle was stretched isotonically during a 7 sec tetanus. After 1 sec of isometric contraction the isotonic lever was released and the muscle stretched 3 mm by a load of 1·3–1·5 times the isometric tension. The load had been adjusted previously so that this stretch occupied most of the remaining 6 sec of the tetanus. Both tension and length were recorded simultaneously so that the work done on the muscle could be calculated.

Under these circumstances, as Abbott & Aubert (1951) first demonstrated, the heat production is roughly the same as during an isometric tetanus of about the same duration, despite the fact that work equal to approximately 30 % of the heat had been absorbed. In my own thirteen experiments the heat had 97 % (± 8 % S.D.) of its isometric value and (heat − work) was reduced to 73 % (± 3½ % S.D.) of that found in a 7 sec isometric tetanus.

As shown by the open triangles in Fig. 5, when (heat − work) is plotted against ΔPC the points lie among those derived from all the other experiments, though perhaps, not surprisingly, their scatter is somewhat greater. On the other hand, if heat alone is plotted against ΔPC (not shown in Fig. 5 but results given in Table 1) the points from the stretch experiments fall significantly above the array of other points. This excludes the possibility that the heat-producing chemical reactions proceed as before and that the work is absorbed without thermal accompaniment.

The general qualitative conclusion to be drawn from Fig. 5 is that all the points seem to be part of the same array about a straight line passing through the origin.

Statistical treatment

The problem remains of giving this qualitative conclusion a quantitative justification. The difficulties that arise in analysing any situation where both x and y variables contain errors have been emphasized earlier (Carlson *et al.* 1967). As a result of that experience it has been possible to arrange the present series of experiments in such a way that some of these difficulties are circumvented. In the earlier work we did not feel justified in assuming that the energy output of the muscle was simply directly proportional to the phosphorylcreatine broken down, partly because we suspected that some of the heat measured was coming directly from the stimulating current rather than from chemical break-down. The statistical analysis of our results confirmed this suspicion but supported the view that the remainder of the heat *was* directly proportional to the PC split. This view is strongly supported by Figs. 4 and 5. Care had been taken to eliminate the heating effect of the stimulus, and also to have a wide spread of both variables so as to demonstrate the functional relation between them as clearly as possible. It is plain in both figures that the points are well fitted by a line that passes through the origin without an intercept on either axis. Statistical analysis then becomes very much simpler. However, it would clearly be incorrect merely to fit to Fig. 5 regression lines that were constrained to pass through the origin, for the scatter increases with the distance from the origin and a least-square procedure would therefore give quite undue weight to the upper points.

Two procedures have been tried for normalizing the errors. The first is to calculate for each individual experiment the value of the ratio (heat + work, mcal)/(ΔPC, μmoles) thus providing a large number of estimates of $-\Delta H$, the molar heat, or, strictly speaking, the molar enthalpy, of hydrolysis of PC. These values are plotted in Fig. 6, which shows that the scatter has become independent of the amount of PC break-down. The second approach, illustrated in Fig. 7, is to plot log (heat + work) against log ΔPC, which also causes the scatter to become uniform. A choice between these two procedures should really be based on which of them leaves the more Gaussian distribution of residual errors, but there is no perceptible difference in this respect, so both have been used.

The next problem is to decide whether, in the different types of contraction, energy output and PC break-down bear the same relation to one another. The results, based on individual estimates of ΔH, are summarized in Table 1. It is clear that the first four groups do not differ significantly from one another so they may be lumped together to give a mean value of $11{\cdot}10 \pm 0{\cdot}23$ kcal/mole ($\pm$ S.E., $n = 52$).

The experiments with stretches show twice as much scatter as the others

and the mean value is high, 12·73 kcal/mole. However, a *t*-test shows that this difference from 11·10 kcal/mole is not significant ($P \approx 0{\cdot}15$) if due account is taken of the difference in size and standard deviation between the two groups: the effect is to reduce the number of degrees of freedom from an expected $52+13-2 = 63$ down to approximately 13 (see *Documenta Geigy Scientific Tables*, 1962, p. 172). However, if in the stretch

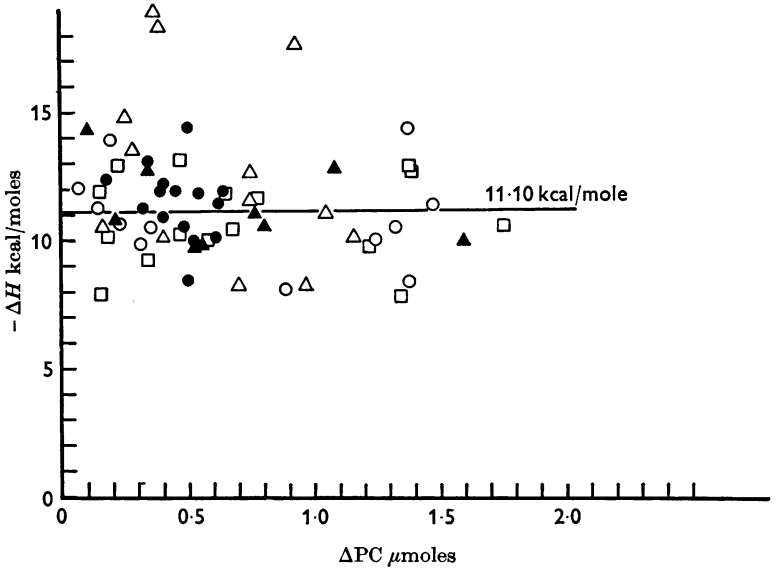

Fig. 6. Ordinates: individual ratios (heat + work, mcal)/(PC split, μmoles). Each point thus gives an estimate of $-\Delta H$, the *in vivo* enthalpy change of PC splitting, in kcal/mole. Abscissae: total PC split, μmoles. The same experiments and symbols as in Fig. 5: ○ ● isometric twitches; □ isometric tetani; ▲ isotonic twitches, positive work; △ stretches, negative work.

experiments ΔPC is compared with heat only, rather than with heat + (negative) work (see last column of Table 1), the difference is significant with $P \approx 0{\cdot}015$.

A further question is whether or not the value of ΔH depends on the extent of the chemical reaction that has occurred. Such an effect might well arise if secondary reactions other than PC splitting are provoked by large amounts of muscular activity. The points at the right-hand ends of Figs. 6 and 7 do show a slight tendency to fall but this is not significant (correlation coefficient $= -0{\cdot}17$) and ΔH can thus be considered to be constant.

In arriving at the best estimate for ΔH the stretch experiments have been omitted because their large standard deviation would give them undue weight in deciding the final answer: the straight lines in Figs. 5 and 6 indicate the result $-11{\cdot}10 \pm 0{\cdot}23$ kcal/mole (S.E. $n = 52$).

In a double logarithmic plot such as that of Fig. 7, the points should lie about a line of unit slope if (heat + work) is directly proportional to ΔPC; and they clearly do so. The correlation between the two variables is very high, with $r = 0{\cdot}98$, if the stretch experiments are omitted. The line of unit slope has been positioned by a least-squares procedure and its intercept gives an estimate of $-\Delta H = 11{\cdot}00 \pm 0{\cdot}23$ kcal/mole ($\pm$ S.E., $n = 52$; as before, the stretch experiments have been omitted). This is very similar

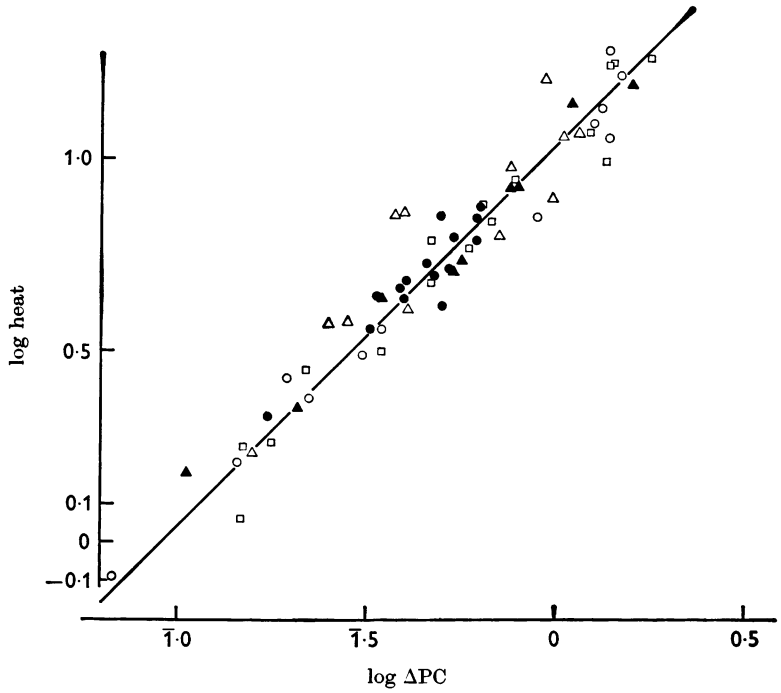

Fig. 7. Logarithmic plot. Ordinates: $\log_{10}$ (heat + work, mcal). Abscissae: $\log_{10}$ (PC split, μmoles). The line has been drawn with unit slope through the grand mean. This is also the position determined by least-squares. The same experiments and symbols as in Fig. 5: ○ ● isometric twitches; □ isometric tetani; ▲ isotonic twitches, positive work; △ stretches, negative work.

TABLE 1. Summary of individual estimates of $-\Delta H$ kcal/mole, as in Fig. 6

	Isom. twitches ○	30 Isom. twitches ●	Isom. tetani □	Isot. positive work ■	Stretches negative work △	Stretches heat only
Mean	10·86	11·47	10·78	11·30	12·73	16·52
S.E.	0·55	0·36	0·43	0·54	1·03	1·37
Coeff. of variation (%)	17·6	12·3	15·8	14·4	29·1	30·0
n	12	15	16	9	13	13

The symbols are the same as those used in Figs. 5–7.

to the previous estimate: the two procedures correspond to taking the arithmetic and the geometric means respectively of the individual ratios of (heat + work)/ΔPC. In the remainder of this paper, for simplicity, the calculations will be based on the value 11·0 kcal/mole.

The weight and water content of muscles

In a previous paper (Carlson *et al.* 1967) it was pointed out that the weight of a muscle is in many ways an unsatisfactory measure of its size. In chemical experiments like the present ones in which the muscle is frozen as rapidly as possible after removal from the thermopile, the drained, or rather frozen, weight inevitably includes a large, and no doubt variable, amount of Ringer fluid. It was suggested that the dry weight (mostly protein) or the total creatine content might provide a better indication of the amount of contractile material present. The creatine content can be measured easily enough but the dry weight can obviously not be determined directly on a muscle that has been extracted with perchloric acid. An attempt was therefore made to estimate dry weight indirectly from the weight of the residue left behind on the filter paper at the end of the extraction procedure. After previous drying and weighing, each filter paper plus debris was dried to constant weight (24–48 hr) over fresh silica gel. The papers cannot be dried in an oven at 105° C because the papers char—apparently as a result of their previous exposure to perchloric acid—and the weight declines progressively.

Figure 8 shows the relation in paired muscles between the *residue weight* and the *dry weight* (oven at 105–110° C, to constant weight). Clearly the two estimates are closely correlated ($r = 0{\cdot}975$, $n = 18$). Probably the residue weight contains greater errors than the dry weight, so the true functional relation is closer to the less steep regression line. Negligible errors will arise therefore if the intercepts are disregarded and it is taken that

$$\text{(residue weight)} = 0{\cdot}926 \times \text{(dry weight)}.$$

Another point raised by Carlson *et al.* (1967, pp. 211–12) concerns the relationship between the wet weight and the dry weight of muscles, which were found not to be simply proportional to one another. The graph relating these two variables, when extrapolated, was found to have an appreciable intercept on the 'wet weight' axis; it was suggested that this might be because the relationship was curvilinear. This could not be tested at the time because the measurements covered too narrow a range to display the form of the function.

Figure 9 is the result of a wider survey which included semitendinosi and gastrocnemii as well as sartorii, and which shows that the relationship is indeed curvilinear, so that extrapolation from the region where wet

weight = 70–140 mg would give an intercept of about 15 mg on the vertical axis, as found before. One way of accounting theoretically for these observations is to suppose that all the muscles have a surface layer of Ringer fluid of fixed thickness which would make a relatively greater contribution to the smaller muscles because of their greater surface to volume ratio. Assuming that all the muscles are similar in shape, which is not too far from the truth, the equation relating wet weight W_t and dry weight W_d is

$$W_t = k_1 . W_d + k_2 . W_d^{\frac{2}{3}}.$$

Fitting this equation to the points in Fig. 9, the values of the constants indicate a surface film roughly 150 μ in thickness, which seems rather large, and without further experimental work this theory should be regarded as a suggestion only.

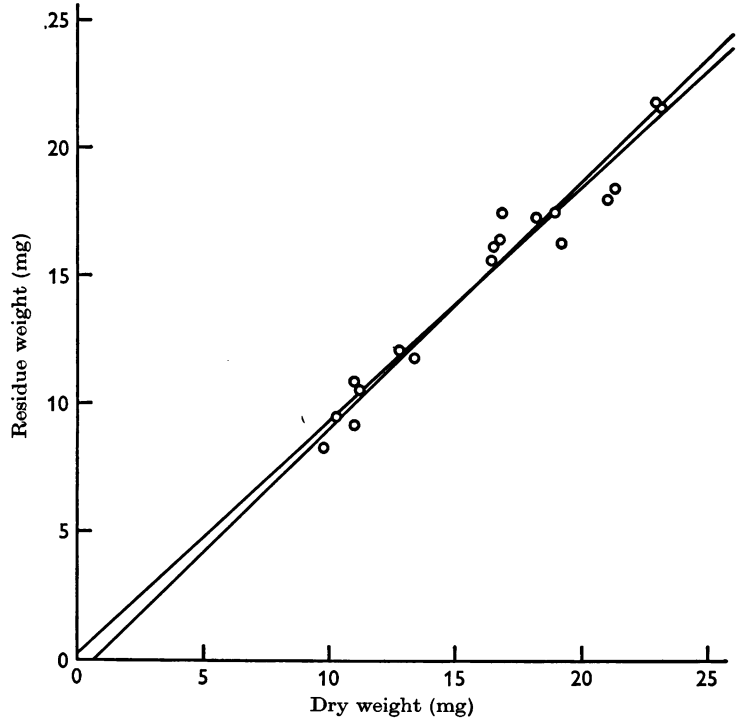

Fig. 8. The relation between residue weight and dry weight in eighteen pairs of frog sartorii. Ordinates: residue weight, mg, see text. Abscissae: dry weight, mg, oven at 105–110° C, to constant weight. Both regression lines are drawn. Not poisoned with IAA.

Returning to the subject of the residue weight, W_r, it can, as we have seen, be used to predict the dry weight. However, the question remains, is it in fact more closely related than is the wet weight (or frozen weight,

W_f) to the chemical composition and contractile activity of the muscle; i.e. is it a better measure of the 'size' of the muscle? This was tested using the available measurements of total creatine content, C μmoles, in the 132 experimental and control muscles. The creatine content turned out to be very poorly correlated with either W_r (r = 0·56) or W_f (r = 0·60). This was puzzling because in short runs of experiments, such as the sets

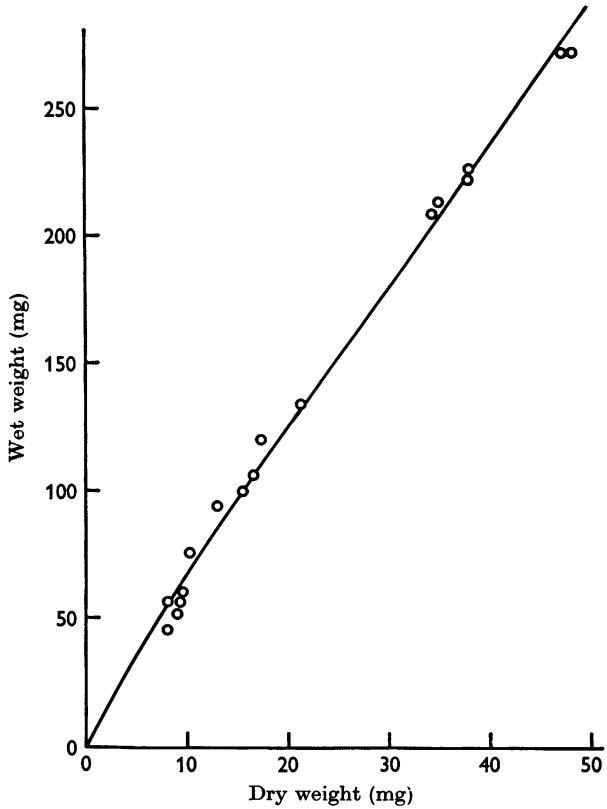

Fig. 9. The relation between wet weight and dry weight in frog semitendinosi, sartorii and gastrocnemii. Ordinates: wet weight, W_t, after draining but not blotting, mg. Abscissae: dry weight, W_d, oven as in Fig. 6, mg. The line has been drawn from the equation: $W_t = 4{\cdot}43\ W_d + 4{\cdot}93\ W_d^{\frac{3}{4}}$. Not poisoned with IAA.

of thirty isometric twitches shown in Fig. 4, the correlation was much higher (r = 0·886 and 0·850 respectively, see Table 2). The discrepancy was finally traced to a very marked seasonal effect, for when the 132 muscles were divided up into five groups according to the time of year, the correlation within each group was much higher (mean values of r, 0·74 and 0·73 respectively) and the functional relationship could be seen to depend on the time of year, the creatine content rising more steeply with weight

during the summer. However, there was clearly no support for the view that W_r was superior to W_f as a measurement of the size of the muscle.

This conclusion was confirmed by a final set of calculations based on the sets of thirty twitches shown in Fig. 4, in which W_r and W_f were compared not only with the creatine content but with four variables all of which might be expected to increase with the size of the muscles. The fifteen correlation coefficients between the six variables are shown in Table 2,

TABLE 2. The correlation coefficients between various measurements of a muscle's 'size'. The results are taken from the fifteen experiments on sets of 30 isometric twitches shown in the second column of Table 1. The mean value of each row is shown in the right-hand column. The small table at the bottom shows the level of significance of the various correlation coefficients shown in the body of the table

	Residue weight	Total mcal heat	ΔPC μmoles	Frozen weight	Total creatine μmoles	Total tension	mean
Residue weight	—	0·640	0·641	0·902	0·886	0·738	0·761
Total mcal heat	0·640	—	0·889	0·722	0·694	0·886	0·767
ΔPC μmoles	0·641	0·889	—	0·694	0·792	0·907	0·785
Frozen weight	0·902	0·722	0·694	—	0·850	0·792	0·792
Total creatine (μmoles)	0·886	0·694	0·792	0·850	—	0·839	0 812
Total tension	0·738	0·886	0·907	0·792	0·839	—	0·832
P	0·02	0·01	0·001				
Correlation coeff.	0·592	0·641	0·760				

which has been arranged in such a way that the correlation coefficient tends to increase from top to bottom and from left to right; this is shown most clearly by the column of means on the right-hand side. It can be seen that the total tension developed is in fact the most closely related to the other variables and is thus the best measure of a muscle's 'size'. The total creatine content (proposed by Carlson, 1963) is next best, and the frozen weight is somewhat better than the residue, or dry, weight; so it seems that there is no point in going to the extra trouble if measuring the last.

DISCUSSION

The main conclusion to be drawn from these results is that no matter what the mechanical conditions of contraction may be, the break-down of phosphorylcreatine over the whole cycle of contraction and relaxation is always directly proportional to the sum of the heat and the work produced by the muscle. The most likely explanation of this fact is that PC splitting is the principal source of energy in muscles poisoned with IAA and nitrogen at 0° C, and that if other reactions do occur to an appreciable extent (see Carlson *et al.* 1967, pp. 224–6) it must so happen that their total amount is also directly proportional to the energy output of the muscle over a

wide range of experimental circumstances. Probably the contribution of such reactions is small—for example, it was found by enzymic analysis (see Bergmeyer, 1963, p. 246) that the formation of fructose 1:6 diphosphate (a likely reaction in IAA-poisoned muscle even at 0° C) amounted to $0{\cdot}40 \pm 0{\cdot}08$ μmoles/g (mean $\pm$ S.E., $n = 7$) following a series of thirty twitches. This is about 8 % of the corresponding PC break-down. Unfortunately the value of ΔH for this reaction does not seem to be known, though its $\Delta G'$ is given by Burton (1957, 1958) as $-4{\cdot}6$ kcal/mole. Taking this as a rough estimate for ΔH, the contribution of this reaction to the total enthalpy change would be only about 3 %.

The constancy of the relation between PC break-down and the enthalpy change of the muscle suggests that over the whole cycle of contraction and relaxation we are concerned with a single chemical process whose extent can be varied either by altering the duration of activity or by altering the mechanical conditions under which contraction takes place. Of course, it does not follow from this that *during* contraction the break-down of PC (or of ATP in muscles poisoned with FDNB) must follow the enthalpy change of the muscle from instant to instant. Recent work by Davies, Kushmerick & Larson (1967) does indeed suggest that early in contraction there has been no chemical break-down during a period when it might be anticipated that there had been a substantial change in the enthalpy of the muscle. However, this discrepancy must have disappeared before the end of the contractile phase, since Mommaerts & Wallner (1967) have shown that no chemical break-down occurs during relaxation.

The effects of positive and of negative work in increasing (to 125 % of the isometric value) or reducing (to 70 % of the isometric value) both the enthalpy change and the PC break-down, have been described before (for references see Davies, 1965 and Wilkie, 1966); the present experiments show in addition that the quantitative relation between these two variables, nevertheless, remains unchanged. In previous work on muscles performing positive work (Carlson *et al.* 1963) there was a worrying discrepancy between the results of purely chemical studies, which showed only 5·9 kcal/mole of work performed, and balance studies similar to those reported here, which showed 9·8 kcal work/mole. The present experiments strongly support the previous balance studies in showing that all types of energy expenditure are 'charged for' at the same rate. Maréchal (1964, p. 110) also concludes that work causes PC break-down at the rate of about 11 kcal/mole.

Experiments on positive and negative work illustrate the close coupling that exists in muscle between mechanical and thermal events. However, the particular form of coupling that is seen in frog sartorii at 0° C may not be of universal significance since it is known that the increase in energy

production when work is performed (Fenn effect) is not shown by some other types of muscle, e.g. frog gastrocnemii at 0° C (Hill, 1965, p. 144); or even frog sartorii at room temperature (Fischer, 1928). The suppression of chemical reaction by stretches has also not been demonstrated to be a universal property of all kinds of muscle. It is important to distinguish clearly between the suppression of chemical reactions and reversal of them (Wilkie, 1966, p. 28).

Comparison with previous results

Resting muscles. The resting PC level in the present experiments, expressed as PC/C, was 0·845. It is clearly similar to previously published values (see Table 3). However, it should be noted in this Table that the standard errors are relatively small, so that several of the values are

TABLE 3. A. Values of PC/C for resting muscles from various sources: in rank order

Source	Date	Mean PC/C	S.D.	S.E.	Number of muscles
Carlson, Hardy & Wilkie	1967	0·889	0·039	0·0053	55
Present work	—	0·845	0·051	0·0064	65
Carlson & Siger	1960	0·840	0·047	0·0081	34
Carlson & Sandberg	1966	0·818	0·038	0·0070	30
Dydyńska & Wilkie	1966	0·785	0·075	0·0137	30
Maréchal, p. 71	1964	0·708	0·020	0·0021	92

B. Comparison between two sets of experiments on 30 twitches

Mean value of	ΔPC μmole/g	$[PC/C]_E$	$[PC/C]_C$	C_E μmole	Total heat (mcal/g)
Carlson, Hardie & Wilkie, 1967	7·98	0·616	0·902	2·515	67·2
Present work	5·00	0·615	0·834	2·079	56·6
Mean value of	Fl/Heat	$\frac{F_{max} \times l}{W}$	ΣF, g wt.	ΔH kcal/mole	n
Carlson, Hardy & Wilkie, 1967	11·5	1·25	1029	11·9	13
Present work	12·0	1·00	878	11·5	15

Notes: Maréchal's value would have been about 0·72 if he had corrected for the formation of creatinine. Subscripts E and C refer to experimental and control muscles. F is the peak isometric force developed in each isometric twitch. F_{max} is the greatest value of F observed in a series of twitches: usually it is observed in the second twitch. l = muscle length; W = frozen weight $\approx$ 1·12 × blotted weight.

significantly different from one another. The reason for this is not clear, though here, too, seasonal effects may play a part. In the present series of experiments PC/C was lowest in January and February (0·805) and highest in March and April (0·871), the difference being significant at $P \approx 0{\cdot}05$.

Active muscles. Fifteen of the experiments in the present series were performed on sets of thirty isometric twitches so as to provide a direct comparison with the similar experiments by Carlson *et al.* (1967). The result, as seen in the lower part of Table 3, is rather puzzling because the PC

break-down in μmole/g is so much smaller in the present experiments. Admittedly tension development and heat production are somewhat reduced also, which could arise merely from a greater water content in these muscles. Another contributory cause may be that within the present series of experiments ΔPC is not directly proportional to muscle weight. The graph relating these two variables has a large intercept and the mean regression line has a slope of 6·8 μmole/g, quite close to the previous value. Whatever the explanation, it is some comfort to find that the estimates of ΔH are not very different in the two series.

The heat of hydrolysis of phosphorylcreatine

From the present work the best estimate for the *in vivo* heat of hydrolysis of PC has been taken as $-11{\cdot}0 \pm 0{\cdot}23$ kcal/mole ($\pm$ S.E., $n = 52$). This is reasonably close to the value $-10{\cdot}6$ kcal/mole derived from the earlier studies of heat production and chemical break-down by Carlson *et al.* 1967. In comparing these values with those derived from *in vitro* measurements (Gellert & Sturtevant, 1960; see also Carlson & Siger, 1960) the great difficulty is to know how much heat to ascribe to secondary reactions with the buffer system present in the muscle, since each mole of PC splitting at about pH 7 needs 0·45 equiv of acid if the pH is to remain constant (Meyerhof, Schulz & Schuster, 1937). The variation of internal pH is small, less than 0·1 pH unit (Caldwell, 1958; Distèche, 1960), so the internal buffers—phosphate, bicarbonate, protein and perhaps carnosine—must be providing nearly 0·45 equiv of acid/mole of ΔPC. The heatproduced from this process over the whole cycle of contraction and recovery (in an unpoisoned muscle) after which the muscle ends up at its original pH, would be $0{\cdot}45 \times 13{\cdot}7 = 6{\cdot}15$ kcal/mole of ΔPC, since by then 0·45 mole of water would have been formed from its ions (ΔH for this process, 13·7 kcal/mole). However, what fraction of this heat appears during the initial pH change, and what fraction during the subsequent neutralization differs from one buffer system to another and is not known with any certainty for the mixed system of buffers found in muscle.

In this connexion it is particularly interesting to note that Meyerhof & Schulz (1935, p. 298) who were well aware of these problems, obtained the value $-11{\cdot}0$ kcal/mole from direct calorimetric measurements on PC splitting *in vitro* in the presence of muscle extract which may have provided the same buffers as during life. The same value is given by Ennor & Morrison (1958). The somewhat lower value, 9·65 kcal/mole proposed by Gellert (see Carlson & Siger, 1960) for 'physiological' conditions, was based on assumptions about the buffer system rather than direct measurements on it.

Practical applications of the estimate of ΔH

Having obtained, with some labour, what is hoped to be an accurate estimate for the *in vivo* ΔH of PC splitting, it is natural to ask what use can be made of it. The following applications have been made so far:

1. In balance studies on recovery from activity in unpoisoned muscles. In normal muscles other reactions than PC splitting occur, notably the formation of lactic acid. In order to estimate the *in vivo* ΔH for a second reaction, it is essential to have an accurate estimate of ΔH for the first one (see Wilkie, 1967).

2. The in vivo ΔH of ATP splitting. An earlier attempt was made to measure this directly by measurement of the heat produced and ATP split in FDNB-poisoned muscles (Dydyńska & Wilkie, 1966). The attempt was foiled by the fact that during the 30 sec or so that are needed to dismantle the thermopile, a number of secondary reactions occurred. It was later realized that ΔH for ATP splitting could be estimated indirectly once the corresponding value for PC splitting was known, if it were possible to compare the heat production in two mechanically identical contractions before and after poisoning with FDNB. The initial break-down of ATP would presumably be identical in the two cases—say n μmoles— but in the unpoisoned muscle the net break-down would actually be of n μmoles of PC, as a result of transphosphorylation.

Then $$\frac{\text{heat produced before FDNB}}{\text{heat produced after FDNB}} = \frac{\Delta H \text{ for PC}}{\Delta H \text{ for ATP}}.$$

However, after poisoning the contractions are often not so vigorous as they were before and a direct comparison of this kind has not yet been made. The fact that the ratio of heat to tension is not appreciably altered by FDNB (Dydyńska & Wilkie, 1966, p. 763) makes it likely that similar contractions would have similar heat production and thus that

$$\Delta H \text{ (ATP)} \approx \Delta H \text{ (PC)} = -11{\cdot}0 \text{ kcal/mole}.$$

No doubt experiments aimed specifically at this point, rather than the incidental observation alluded to, could produce more accurate information. On other grounds, George & Rutman (1960, Table 11) also conclude that the Lohmann reaction should be almost thermoneutral.

3. The efficiency of contraction. It remains a melancholy fact that we still do not know the efficiency of the contractile process. Eighty per cent of the free energy obtainable from oxidation of substrate is undoubtedly wasted, despite what must have been powerful evolutionary pressure; but what fraction of this waste occurs during contraction and what fraction during recovery remains unknown (see Wilkie, 1968). In order to calculate the efficiency of contraction it is necessary to know the ratio $\Delta G/\Delta H$ for

PC splitting (Wilkie, 1960, p. 271) and it is at least a beginning to know what ΔH is. The uncertainty surrounding ΔG has been described recently by Wilkie & Woledge (1967) and will not be discussed further here. One interesting fact that has been published since that paper was written is the experimental finding by Davies *et al.* (1967) that a muscle poisoned with FDNB can produce 5·5 kcal of mechanical work/mole of ATP split. It follows that ΔG for ATP splitting and for PC splitting must be at least $-5{\cdot}5$ kcal/mole. We can also derive an indirect estimate of ΔH for PC splitting, for in a normal unpoisoned muscle we must be able to obtain at least 5·5 kcal work/mole of PC split, by the argument given under (2). Suppose that n moles of PC have been split, then

$$w_{\max}+h = n\times(-\Delta H) \quad \text{and} \quad w_{\max} = 5{\cdot}5\times n.$$

For contractions of normal muscle, Hill (1964) has shown experimentally that $w_{\max}/(w_{\max}+h)$ can rise to 0·45, thus $5{\cdot}5\times n/n\times(-\Delta H) = 0{\cdot}45$ so $\Delta H = -12{\cdot}2$ kcal/mole, which is not in too bad disagreement with the value of 11·0 kcal/mole obtained in the present work.

4. Calculation of the number of rephosphorylations obtained (m) per hexose unit oxidized. In a normal muscle undergoing a brief contraction followed by recovery in oxygen, the over-all reaction is:

	Change of enthalpy (kcal)
$C_6H_{10}O_5+6O_2 = 6CO_2+5H_2O$	-700

(all quantities are expressed per hexose unit)

This over-all reaction can be divided into two parts:

during contraction phosphorylcreatine, PCr, breaks down

$$m\text{PCr} = m\text{P}_i+m\text{Cr} \qquad -m\times 11{\cdot}0$$

and during recovery it is rebuilt

$$C_6H_{10}O_5+6O_2+m\text{P}_i+m\text{Cr} = 6CO_2+5H_2O+m\text{PCr} \qquad -(700-m\times 11{\cdot}0)$$

These changes of enthalpy can be related to myothermic measurements of initial and recovery heat. It is usually assumed, though without direct analytical confirmation, that by the end of the oxidative recovery heat production (after about 40 min at 0° C) the only net reaction has been the oxidation of a small quantity of substrate, all the other intermediary metabolites being back at their normal resting levels. Thus if

$$\frac{\text{enthalpy change during recovery}}{\text{enthalpy change during contraction and relaxation}} = R,$$

$$R = (700-m\times 11{\cdot}0)/m\times 11{\cdot}0.$$

For frog muscle at 0° C, $R = 1{\cdot}0$ (Hill, 1965, p. 183), so $m = 32$. Textbooks of biochemistry, whose conclusions are based mainly on mammalian

material, usually give 39 as the value of m. The discrepancy is not large, and may well be genuine. It is hoped that further work in progress on unpoisoned muscles may shed more light on this question.

I wish to express my deep gratitude to Mrs Claude Gilbert for all the hard work that she has cheerfully and conscientiously done in the course of this research. I should also like to thank the Medical Research Council for supporting her financially.

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